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Stepwise Extraction of Proteins and Carbohydrates from Soybean Seed

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The stepwise hot water extraction of soybeans, which were extractions in a series of procedures of whole soybean seeds, dehulled and sliced ones, and pressed ones carried out by autoclaving, was investigated to study the localization in the seed and their characteristics. The characteristics of each extraction were studied by HPLC, SDS−PAGE, components analysis, microscopic observation, and effect for some enzymes. Carbohydrates were easier to extract than protein. In the extractions, the ratio of uronic acid per total sugar was constantly about 0.3. A comparison of these extracts, soybean milk, extraction from defatted soybean meal, and soybean milk residues was also carried out, and the characteristics and the localization were investigated. Mid-sized proteins in soybean milk were easy to extract. However, hardly any high molecular weight proteins or high molecular weight carbohydrates were extracted. The proteins and carbohydrates were considered to be localized in the middle lamella and in the protein and/or oil bodies of the cell, and the proteins and carbohydrates were gradually extracted through seed and cell breaking. Gelation was observed only in the boiled extracts from whole seeds. Pepsin and trypsin digests of the high molecular weight protein had inhibitory activity against the angiotensin I converting enzyme.

KEYWORDS: Soybean; extraction; soybean protein; soybean carbohydrates

INTRODUCTION

Soybean is an oil seed which has good protein. The soybean is defatted, and the residual soybean (meal) is used to produce soy protein or animal feed (*1*−*3*). In soybean meal, the soybean is already milled or pressed, and most of the soybean cells are broken. Therefore, the water extraction is done, and then various proteins and carbohydrates are extracted as a mixture. Methods for the extraction of soy protein such as glycinin and β-conglycinin are well-known (*3*−*5*). Water-extractable soy carbohydrates have been well-studied (*6*−*12*). However, the extraction of protein and carbohydrate from soy meal in high yields is difficult. Many studies have attempted to improve the extraction methods (*6*−*8*, *13*). Recently, Fischer et al. (*6*) reported that heat and humidity are important factors in the extraction of proteins from soybean meal. They showed that the proteins and carbohydrate formed a matrix in the soy meal and that the matrix interfered with enzymatic digestion. The enzymatic extraction was also studied, and attempts were made to improve it. Ouhida et al. reported the extractability from the meal and analyzed the components of the extraction using enzymes or chemicals (*8*). They reported the component analysis of the water extracts by the enzymatic and alkaline gradient extracts and showed that the residue after enzymatic and chemical extraction was mainly cellulosic material.

We have been studying enzymatic digestion of soybean treated by hot water (autoclaving at 121 °C for 10 min) extraction and making of single cells and also the extended enzymatic high digestion for the soybean residue (okara). Our results mainly agreed with those of Fischer et al. (*6*); the proteins and carbohydrates formed a complex, and heating with water was effective in breaking the complex to allow access by enzymes (*14*, *15*). Thus, the enzymatic and chemical extractions have been well-studied to improve the extractability of soy meal. However, few reports have investigated the stepwise extraction of protein and carbohydrate from the raw soybean seed. The localizations of water-extractable proteins and the carbohydrates in soybean seeds are not well-known. The various components of soybean, such as extractable and/or unextractable substances and the oil, are functionally arranged in each cell.

Soybean meal and soybean seed would have essentially the same components except soybeans contain oil and the soybean meal has broken cells. This information on the localization of proteins and carbohydrates of soybean would make it easier to develop strategies for improving the extractability and enzymatic digestion of soy meal. Soybean is also used to make products such as soy sauce, pickles, and soybean soup. In these products, soybeans are processed or fermented without breaking. Changes in the components of the soybean in the making of these processed foods and during fermentation have not been well-investigated.

Therefore, we think that research from this viewpoint will be important. Knowledge of the localization of the water-

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extractable protein and carbohydrate would be useful for the new soybean processing and its improvement and also for determining the localization of the food function.

In this paper, we report the characteristics of the extractions from the raw soybean by a series of extractions using autoclaving and examine the characteristics of the extractions.

The serially extracted materials including raw soybean with or without a hull, dehulled soybean, and sliced or compressed soybean were investigated. We also report the mutual relations of the defatted soybean, raw soybean, and okara by staining and microscopic observations.

MATERIALS AND METHODS

Extraction from Soybean. Soybeans (*Glycine max L.*), cultivated in the USA, and the soybean meal were gifts from Showa Sangyou Co., Ltd., Tokyo, Japan. Soybean residue (okara) after manufacturing soy milk was a gift from Matsuda Food Co., Inc., Osaka, Japan. The sequential extraction of soybeans from the raw whole soybean, dehulled soybean, soybean cracked into four parts, and pressed soybeans were sequentially done by autoclaving in water. All autoclavings were done at 121 °C for 10 min. For the first extraction, the raw soybeans were mixed with 5 volumes of water and autoclaved. The water extraction was filtered (extraction A). For the second extraction, the autoclaved soybeans were dehulled and autoclaved (extraction B). For the third extraction, the autoclaved soybeans were cracked into four parts and autoclaved (extraction C). For the final extraction, the cracked and autoclaved soybeans were freeze-dried and the dried samples were pressed at 150 kg/cm^2 for 5 min using a jack. The pressed samples were mixed with 20 volumes of water and autoclaved (extraction D).

Estimation of Sugar and Protein. The amount of uronic acid was measured by the 3-phenylphenol method (*16*). The total sugar was estimated by the phenol−sulfuric acid method (*17*). The reducing sugar was estimated by the Nelson−Somogyi method (*18*). Protein was estimated by the Lowry method (*19*). Each amount was calculated using a colorimetric standard curve with D-galacturonic acid, D-glucose, and serum albumin, respectively, as the standards.

HPLC Analysis. The extracted samples were centrifuged at 3000 rpm for 5 min. The freeze-dried samples of extractions A−D were dissolved in 100 volumes of water. A 10 μL aliquot of the supernatant or the dissolved freeze-dried samples was applied to an analytical HPLC system (DP8020, Toso, Tokyo, Japan) consisting of a molecular sizing column (TSK-Gel G3000SWXL, 7.8 × 300 mm, Toso), a difference refraction indicator RI8020 (Toso), and an optical photometer UV8020 (Toso) for detection.

Light Microscopic Observation. The microscopic observations and photographs were done using a light microscope, Olympus Model BH-21 (Olympus Optical Co., Ltd., Tokyo, Japan) and a digital microscope photography device, DP-II.

Staining and Observation of the Okara. *O*-Toluidine was used for staining pectin and lignin. Protein staining was done with acrolein−Schiff reagent. The polysaccharides were stained by PAS dyeing (*20*).

SDS−PAGE Electrophoresis. SDS−PAGE was done by the Laemmli method (*21*). The electrophoresis equipment used was an AE-9631M/P with an attached concentration slope gel (5−20%; manufactured by ATTO, Tokyo, Japan). The standard molecular marker was the Kaleidoscope prestained standards BIO-RAD.

Characteristics of Extracted Samples. *Transparency.* To measure the transparency, a sample solution (1%) was prepared from the freeze-dried sample, and the pH was adjusted to pH 4.5 with 1 N HCl, or $MgCl_2$ was added to a final concentration of 20 mM. The absorbance was measured at 660 nm.

Gelation. Gelation was estimated by the change in the viscosity using a digital viscometer, Visconic Type ELD, Tokyo Rika, Tokyo, Japan.

Inhibition Test for Digestive Enzymes. The inhibition or activation activity of the samples solution (1%) was tested for starch hydrolysis by saliva amylase, caseinolytic activity by trypsin, tributylin hydrolysis by pancreatic lipase, and hippuryl-His-Leu hydrolysis by the angiotensin I converting enzyme (ACE) (*22*). The sample solutions (5%) were digested at 36 °C overnight by 0.5% pepsin or trypsin, and the ability of the digested solutions to inhibit the activity of ACE (1.25 μU) was measured. The ACE activity was measured by the amount of the dipeptide His-Leu using a fluorescence meter, Shimadzu RF-1500, Kyoto, Japan.

Enzymes. The enzymes and their suppliers are as follows: pepsin type, P-6887, porcine stomach mucosa 3276 U/mg, Sigma Chemical Co., St. Louis, MO; trypsin, type IX-S, porcine pancreas, 13130 U/mg, Sigma; angiotensin converting enzyme, from bovine lung, 0.2 U, Wako Pure Chemical, Osaka, Japan.

RESULTS

Permeation HPLC of the Stepwise Extraction from Soybean and Its Characterization. Figure 1 shows the results of the permeation HPLC analysis on the series of extraction samples by autoclaving (extractions A−D), the extract of defatted soybean meal by autoclaving, and the soybean milk by filtered drop. The absorption signals at UV 280 nm were characterized as UV-1−UV-6. The signals of RI were characterized as RI-1−RI-5. All of the UV absorption peaks of extractions A−D were detected in the extraction from the defatted soybean meal. The RI peaks of extractions B−D gave a similar elution profile. **Figure 2** shows the results of the HPLC analysis of the same freeze-dried extraction samples (extractions A−D) and defatted soybean meal. The UV and RI signals are shown by FUV-1−FUV-6 and FRI-1−FRI-6, respectively. The elution profiles (UV-1−UV-6) in **Figure 1** are similar to those of FUV-1−FUV-6 in **Figure 2**. On the other hand, the elution profiles of the RI peaks (FRI-1−FRI-6) were different from RI-1−RI-5 in **Figure 1**. The signal patterns (FRI-1−FRI-6) of the freeze-dried samples were similar to each other.

It was found that the high molecular proteins were newly extracted during the new extracting steps. The effects of the physical destruction such as dehulling, slicing, and pressing were reflected in extractions A−D. A drop of soybean milk filtered through a membrane filter gave peaks similar to those of UV-3, -4, and -5 and small amounts of UV-1 and -2 (**Figure 1F**). As for the freeze-dried samples, the peak of FRI-4 in the middle molecular weight range was recognized in the order of extraction A > extraction C = extraction B > extraction D. The RI peak height of FRI-1, -2 in the high molecular range was found to be in the order extraction D > extraction C > extraction B > extraction A. From the profiles of the RI peaks of the extracts, the FRI-4 and -5 were easily extractable with and without the seed hull and the cracking. The high molecular weight extracts (FRI-1, -2) were more extracted with the extraction steps; the high molecular weight carbohydrates were not easy to extract but were extractable by seed cracking and destruction by pressing.

Characteristics and Identification of RI-4 and -5. In **Figure 1**, the RI-4 of extraction A was especially characteristic. **Figure 3** shows the HPLC analysis to investigate the change of RI-4 of extraction A. RI-4 in the extraction by autoclaving was observed to shift to RI-5 after storage overnight (**Figure 3a,b**). RI-4 was hardly detected in the boiling extraction from soybean; the peak was found in the autoclaved raw soybean with the hull. The RI peak of the same position of elution was not found from part of the outside cotyledon of the soybean and hull (**Figure 3c**). RI-4 was found in part of the inside in the cotyledon of the soybean (**Figure 3d**). RI-4 was also found in extraction of soybean meal by autoclaving (**Figure 3e**). However, an extract from the hull itself did not give RI-5 (**Figure 3f**). Therefore, RI-4 could not be present only in the hull but in the inside cotyledon of the soybean. The retention times of RI-4 and UV-3 were very similar, but only uronic acid precipitated during extraction A after the pH was lowered to 4.5, and protein was not found in the precipitate (**Figure 3g**). RI peaks, RI-4, were detected only in the supernatant of extraction A; no UV

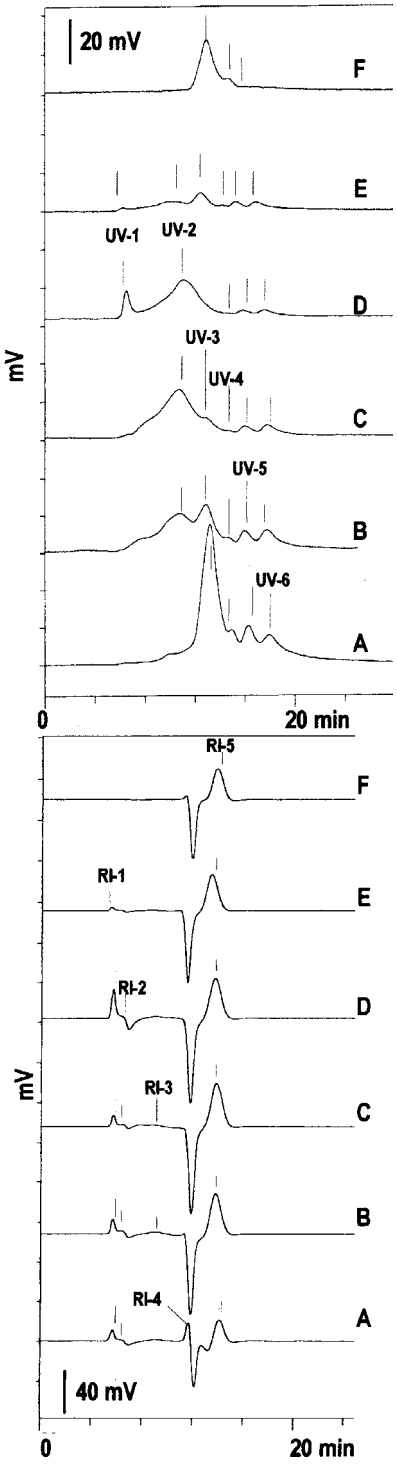

Figure 1. HPLC analysis of a series of extraction from soybean. The samples of the extraction and filtered soybean milk were analyzed by HPLC. Each elution profile of the extracted sample was indicated by abbreviation characters. Symbols: **A**, extraction A; **B**, extraction B; **C**, extraction C; **D**, extraction D; **E**, deffatted soybean meal; **F**, filtered soy milk. The absorbance signals at UV 280 nm (upper elution profile) are shown as UV-1–UV-6, and the signals of RI (lower elution profile) are shown as RI-1–RI-6. The vertical lines in the HPLC profiles represent the identified small peaks of the UV-1–UV-6 and the RI-1–RI-6. Sample size, 10 μL; column, TSK-Gel G3000SWXL (7.8 × 300 mm; Toso, Tokyo, Japan); eluent, 0.1 M phosphate buffer, 0.2 M NaCl, pH 7.0; flow rate, 1 mL/min.

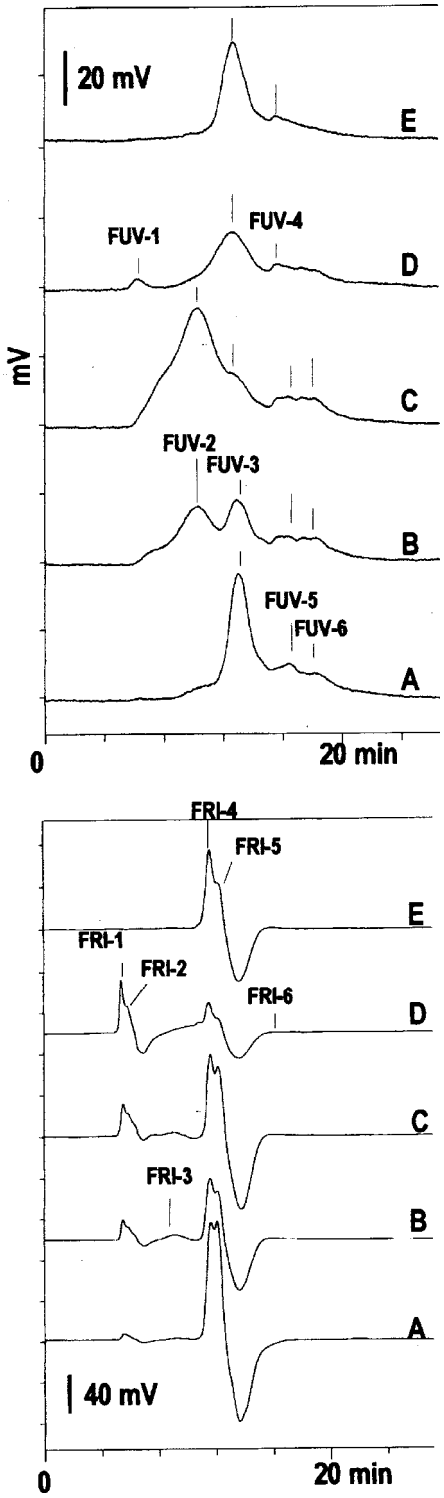

Figure 2. HPLC analysis of freeze-dried series of extraction from soybean. The freeze-dried samples of the extraction and filtered soybean milk were analyzed by HPLC. Each elution profile of the extracted sample is indicated by abbreviations. Symbols: **A**, extraction A; **B**, extraction B; **C**, extraction C; **D**, extraction D; **E**, deffatted soybean meal. The absorbance signals at UV 280 nm (upper profile) are shown as FUV-1–FUV-6, and the signals of RI (lower profile) are shown as FRI-1–FRI-6. The vertical lines in the HPLC profiles represent the identified small peaks of the UV-1–UV-6 and the RI-1–RI-6. Sample size, 1%, 10 μL; column, TSK-Gel G3000SWXL (7.8 × 300 mm; Toso, Tokyo, Japan); eluent, 0.1 M phosphate buffer, 0.2 M NaCl, pH 7.0; flow rate, 1 mL/min.

peak was detected in the water extraction from the residual soybean after extraction of extraction A (data not shown). The ratio of the uronic acid/total sugar was 0.87 (RI-4, -5) and 1.2 (RI-1, -2), respectively. These RI peaks were mainly considered as polygalacturonic acids. These results also suggested that

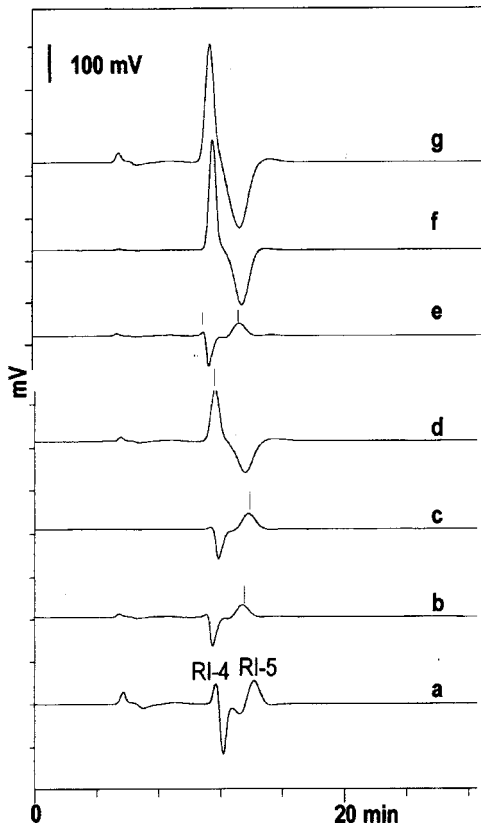

Figure 3. Changes and comparison of RI peaks of the HPLC analysis for extraction A from soybean. The samples of extraction A by different methods or from different parts of the soybean, the extraction of the hull, and the resolved sample of the precipitant of extraction A at pH 4.5 were analyzed by HPLC. Each elution profile of the extracted sample is indicated by abbreviation characters. Symbols: **a**, extraction A by autoclaving; **b**, extraction A by boiling; **c**, extraction A from the outside part of the soybean seed by autoclaving; **d**, extraction A from the inside part of the soybean seed by autoclaving; **e**, extraction of the deffatted soybean meal by autoclaving; **f**, extraction from the hull of the soybean seed; **g**, the resolved sample of the precipitant of extraction A at pH 4.5. The absorbance signals are shown as RI peaks. Sample size, 10 μL; column, TSK-Gel G3000SWXL (7.8 $\times$ 300 mm; Toso, Tokyo, Japan); eluent, 0.1 M phosphate buffer, 0.2 M NaCl, pH 7.0; flow rate, 1 mL/min.

polygalacturonic acid from the parts inside and outside of the raw soybean seed was first extracted by the autoclaved extraction but not extracted from the boiled one.

Sugars and Protein Estimation of the Series of Stepwise Extraction from Raw Soybean. Table 1 shows a composition analysis of the series of extractions from raw soybean. The analysis results of total sugar, total uronic acid, reducing sugar, and protein are summarized. From the results of **Table 1**, the amounts of the extracted protein were increased in each step, but the total sugar decreased. In the extractions, the extractable

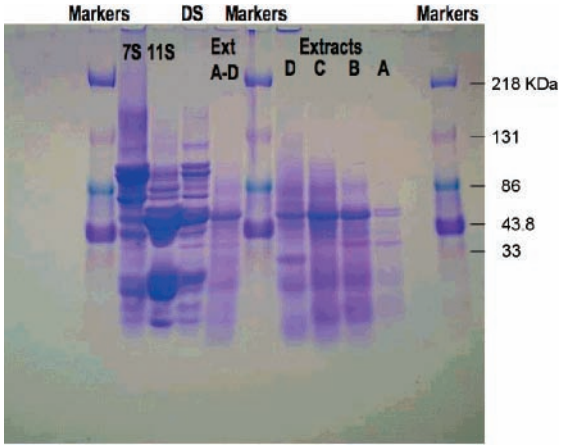

Figure 4. Series of extraction from soybean on SDS–PAGE. The freeze-dried samples of each extraction of soybean (extractions A, B, C, and D; 1%), mixture of extractions A–D (Ext A–D), extraction of defatted soybean (DS), samples of 7S (7S) and 11S (11S) were treated with SDS, and the sample solutions were applied on SDS–PAGE gel. Sample size, 10 μL. Staining was done with CBB.

sugars were easier to extract than the extractable proteins. These results agreed with the results of the HPLC analysis. The ratio of the uronic acids in the extractable sugars of each extraction was almost constant and was fixed at about 0.3. That of the autoclaved extract of only the hull was 0.6.

SDS-Electrophoresis of the Series of Stepwise Extractions. Each extracted fraction was freeze-dried, and the SDS-electrophoresis was carried out. These results are shown in **Figure 4**. Although a small difference from some of the protein bands was found, clear differences were not seen in each fraction. This result did not agree or support the analysis by HPLC. The UV-1 and UV-2 in the HPLC pattern could be the broad, weak stained band with CBB and PAS in the SDS−PAGE (data not shown).

Microphotographs of Each Extraction Stage. The soybean cells after each stepwise extraction were observed with a microscope, and the results are shown in **Figure 5**. In the microscope figure of the dyed cells, all of the PAS dyeing (saccharide), the *O*-toluidine dyeing (the pectin and the lignin), the CBB dyeing (protein), and acrolein−Schiff dyeing (protein) became weak as the stepwise extraction proceeded, except for extraction D. Inner particles of the soybean cells such as protein and oil bodies and the shell including the bodies were found to be shrunk by autoclaving. Most of the proteins of the protein bodies in the soybean cells are soluble. This photograph was considered to be agreeable. The extracted components inside the soybean cell could pass through the cell walls and hulls, and it would be extracted. The protein and sugar components around the cell were also extracted and removed by autoclaving. It was observed in the cell of extraction C that soybean oil was found between the insides of the cell wall and the cell. In the cell of extraction D, the cell wall and inner structure of the cell

Table 1. Compositions of Extractions A–D

	composition (mg/(g of soybean))			
	extraction A[a]	extraction B[a]	extraction C[a]	extraction D[a]
total sugar	62.5 ± 10.14	28.6 ± 2.90	17.9 ± 2.02	5.7 ± 1.09
total uronic acid	20.6 ± 2.57	9.4 ± 1.50	6.2 ± 0.96	1.6 ± 0.24
reducing sugar	3.8 ± 0.56	1.5 ± 0.28	0.6 ± 0.10	0.2 ± 0.12
protein	7.1 ± 0.82	12.6 ± 2.10	18.6 ± 0.52	7.6 ± 5.07
uronic acid/total sugar[b]	0.33	0.33	0.35	0.29

[a] Each value was expressed as av ± SD mg/(g of soybean) (n = 4). [b] The ratio was calculated from the total sugar/total uronic acid in the table.

Table 2. Characteristics of Extractions A–D[a]

	extraction A	extraction B	extraction C	extraction D
transparency[b]	0.041 ± 0.003	0.027 ± 0.013	0.026 ± 0.002	0.088 ± 0.006
pH 4.5[c]	0.40 ± 0.236	9.66 ± 2.405	11.14 ± 3.540	3.16 ± 0.163
20 mM $MgCl_2$ *d*	1.05 ± 0.284	5.56 ± 1.123	1.69 ± 0.706	2.67 ± 0.125
gelation[e]	gelation	ND	ND	ND
enzyme inhibition[f]				
saliva amylase	ND[h]	ND	ND	ND
trypsin	ND	ND	ND	ND
pancreatic lipase	ND	ND	ND	ND
angiotensin converting enzyme[g]				
pepsin digestion	0.5 ± 3.27	10.4 ± 2.09	25.0 ± 9.64	30.2 ± 0.66
trypsin digestion	6.5 ± 5.90	23.8 ± 2.56	33.1 ± 3.86	32.0 ± 0.76
pepsin and trypsin digestion	5.9 ± 8.46	31.9 ± 7.65	41.4 ± 5.03	27.0 ± 2.89
none	ND[h]	ND	ND	ND

[a] Each value was expressed as av ± SD mg/(g of soybean) ($n = 4$). All samples were prepared from freeze-dried extraction; the concentrations were 1%. [b] Transparency was measured at 660 nm. [c] The aqueous solutions were adjusted to pH 4.5 with the addition of 1 N HCl; the optical density at 660 nm was measured. [d] The aqueous solutions were measured at 660 nm containing 20 mM $MgCl_2$; the optical density at 660 nm was measured. [e] The gelation required boiling the solution (>8%) and cooling below 15 °C. [f] The extracts (0.1%) were added to the enzyme reaction mixture. [g] The digested extracts (0.02%) were added to 0.9 mL of the ACE (1.25 μU) reaction mixture. [h] ND means not detected; the inhibition was below 10%.

body were broken by pressing, and it was observed that soybean oil drops inside the cell were extracted, and the structure in the cell was well-stained compared with the cells of extraction C and extraction D.

Comparison of HPLC Analysis with the Soybean Residues (Okara) and Extraction C. Figure 6 shows the result of the HPLC analysis. Overlaid UV profiles of extractions C and D were very similar to that from okara. Small UV peaks were detected in the extraction from the boiling okara; however, autoclaving could allow more extraction of the high molecular weight proteins from okara. The HPLC elution profile of the extraction gave a similar UV-2 of extraction C. **Figure 7** shows PAS and the acrolein−Schiff dyeing of the soybean residues after the extraction by boiling or autoclaving. The microscopic figures support the results that autoclaving is an effective extraction. In soybean residues, most of the soybean cells were milled, and the inner ingredient was removed. The HPLC and microscopic observation results suggested that the protein of UV-2 would be extracted from the middle lamella between the cells.

Other Characteristics of the Extraction. Table 2 summarizes the characteristics of the extraction series. The turbidity, gelation, and effects for α-amylase from saliva, trypsin, and pancreatic lipase were tested. As for the extract in extractions C and D, it was characteristic to have a strongly white microprecipitation by changing to pH 4.5 and the 20 mM $MgCl_2$ addition. White precipitation at pH 4.5 was stronger than that of $MgCl_2$. The HPLC profile of that supernatant and the redissolution of the precipitance had the same elution profiles (data not shown). A unique characteristic of extraction A was gelation. This gelation activity was found to be more than 8%, and it found that it became remarkable below 15 °C. The autoclaved extract of the hull did not produce a gel and did not harden, although it became a viscous liquid. The inhibition or activation for the saliva amylase, trypsin, and lipases of pancreatin, which are digestive enzymes, were not specially found. Inhibition activity for the angiotensin converting I enzyme of the extracts digested pepsin and/or trypsin were strongly detected in extractions C and D.

DISCUSSION

We investigated the stepwise extracts to determine the components from the soybean seeds using sequential autoclaving. Most of soybean cells except the stage of extraction D were not broken, and the heat water extractions were carried out. Although the soybean cells are broken in the case of the defatted soybean meal or soybean milk, the principle extractions were considered to be similar. Simple autoclaving (121 °C for 10 min) was very effective in removing adhesives of the middle lamella (*14*, *15*). The autoclaving extraction was also effective for the soybean residue from soymilk manufacturing, okara; this result showed that an effective water extraction was not done by only boiling and seed breaking. The HPLC analysis and the microscopic observation agreed with each other. Fischer et al. reported the detailed enzymatic extractability of soybean meal protein and carbohydrates and heat and humidity effects for the extraction. High humidity and heat were essential for the effective extraction with or without enzymes (*6*). The autoclaving temperature and the period, 121 °C for 10 min, is the minimum condition for sterilization and is not difficult. We have reported the preparation of single cells by autoclaving for soybean oil extraction; most of the soybean oil remained in the soybean single cells unless a soybean cell was broken by mechanical means (*15*).

The existence of small amounts of UV-2 in extraction A and extraction B was thought to influence the removal of the hull. The increasing of the UV-2 extraction and the decreasing of UV-3 in extraction C and extraction D were due to the soybean slicing and the sequential extracting. All autoclaved fractions were also detected in the extraction of defatted soybean; this result showed that the extraction was fundamentally considered to be the same and also indicated a physical effect and extraction resistance in the soybean seed. The results were also confirmed by the microscope-dyeing observations. A difference in the quantity ratio of the FRI peaks was found, but a remarkable difference was not recognized as the pattern of the FRI profiles in the freeze-dried extractions. This result shows that molecular species of extractable carbohydrates of the soybean seed would be principally similar, but an interaction of carbohydrates and proteins would occur in the extraction of soybean. On the other hand, the high molecular weight RI peaks (FRI-1, -2 and RI-1, -2) increased. It was shown that the high molecular weight carbohydrates were tightly bound in the inner soybean. The similar result was also found in the extraction of protein. The RI signals were mainly polygalacturonic acid. The changing of the elution profile of RI-4 to RI-5 or the change between RI and FRI peaks was found. The results would imply the extracted carbohydrates and the extracted proteins would be interacted in the extraction. The middle and the low molecular weight

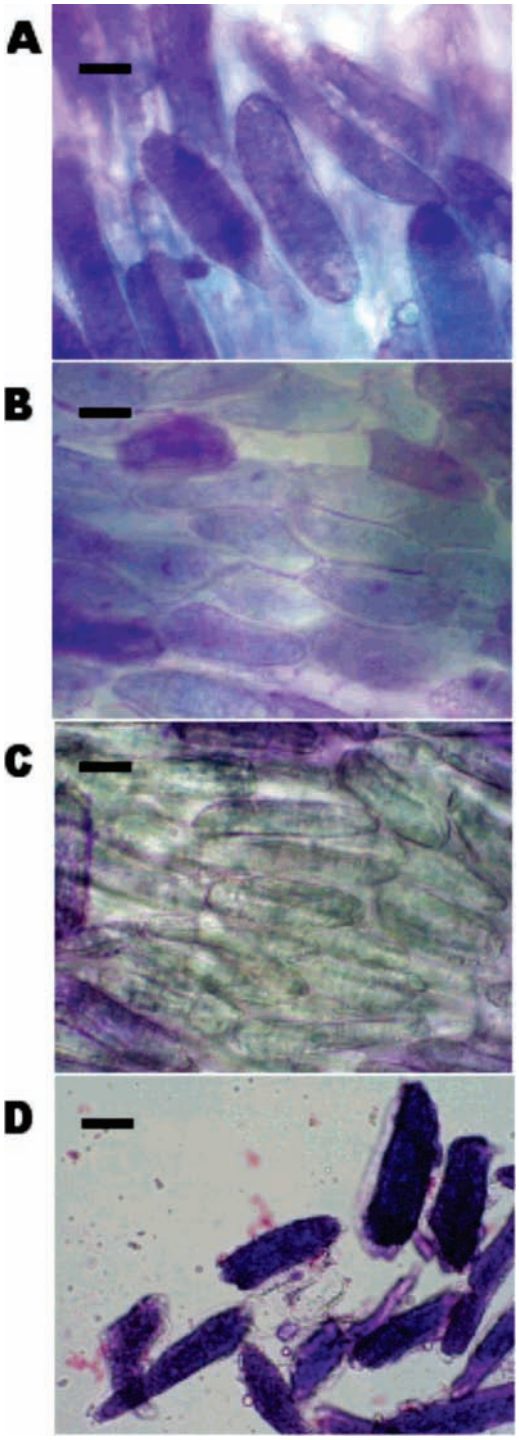

Figure 5. Microscopy of soybean cells in the series of extraction. Photographs from **A** to **D** indicate the sliced samples after the extraction treatment of extractions A–D, respectively. The cells after treatment of extraction D were directly dispersed in water. Stainings were done by *O*-toluidine. The black bar represents 10 μm.

proteins of UV-3−UV-6 in extraction A were clearly found in the membrane-filtered drop from the soybean milk, but UV-1 and -2 were a very small amount. Moreover, for the comparison with the soybean residues, okara, the water extract of okara by autoclaving gave results similar to that of UV-2 of extraction C. This peak, UV-2, was slightly detected in the extraction by boiling but well detected in the extraction by autoclaving. The microscopy observation (Figure 6) showed that UV-2 would be extracted from the surrounding of the soybean cells (middle lamella between cells).

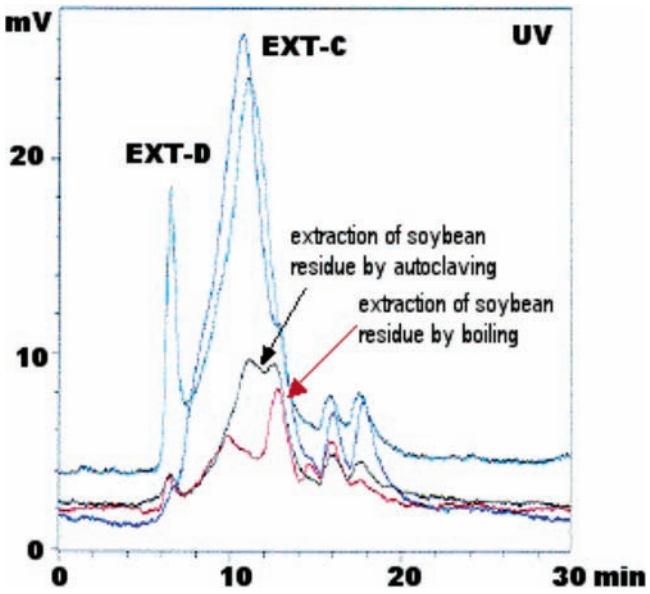

Figure 6. Comparison of HPLC analysis of extraction from soybean residue, okara. The sample of soybean residue, okara, was extracted by boiling or autoclaving in water (121 °C, 10 min). The supernatant samples were injected on the HPLC column, and analyzed. Autoclaved extraction was more effective for the extraction than the boiling process, and the components of UV-1–UV-6 were detected. The UV-2 was significantly extracted by autoclaving.

Comparing micrographs of soybeans from extraction C and extraction D, the secondary cell wall was broken by compressing and the inner soybean oil drops were extracted. The structured shell or envelope including oil and protein bodies was clearly stained with *O*-toluidine solution in the cell of extraction D. A high molecular weight protein (UV-1) was newly extracted in extraction D. The UV-1 was also detected in the extraction from soybean meal, and it was considered that this protein could not go through the shell of structured bodies inside the cell or the secondary cell wall. However, the knowledge of UV-1 localization in the inner shell of the bodies could not be clarified. Though the UV-1 was hardly observed in the filtered soybean milk drop, the high molecular weight protein of UV-1 and -2 were found in the extract from the pressed soybean (extraction D). In the soybean milk, the high molecular weight protein of UV-1 and -2 would hardly be extracted from the milled soybean by boiling or steaming. The high molecular weight proteins and carbohydrates existed in or around the soybean cells and were extracted by only heated water. It is important for the effective extraction of the carbohydrates and the proteins from soybean that the soybean seed should be finely crashed to break the adhesion of cells and the cell itself, and the extraction should be done with not boiling water but the heated water such as autoclaving. The breaking the cells should be done before extracting, because the cells were softened by the autoclaving (*15*). The physical destruction of the soybean seed itself and the soybean cell was necessary to obtain the high molecular weight proteins and carbohydrates, and heated water such as autoclaving was also necessary for the effective extraction. The humidity and heat treatment are essential for effective extraction (*6*). A further study of the characterization and the localization of each component will be needed.

The condensed extraction A from the soybean with boiling resulted in gelation. This gelation would be due to the coexistence with polygalacturonic acid and the protein from extraction A. We also have obtained gelation by condensed

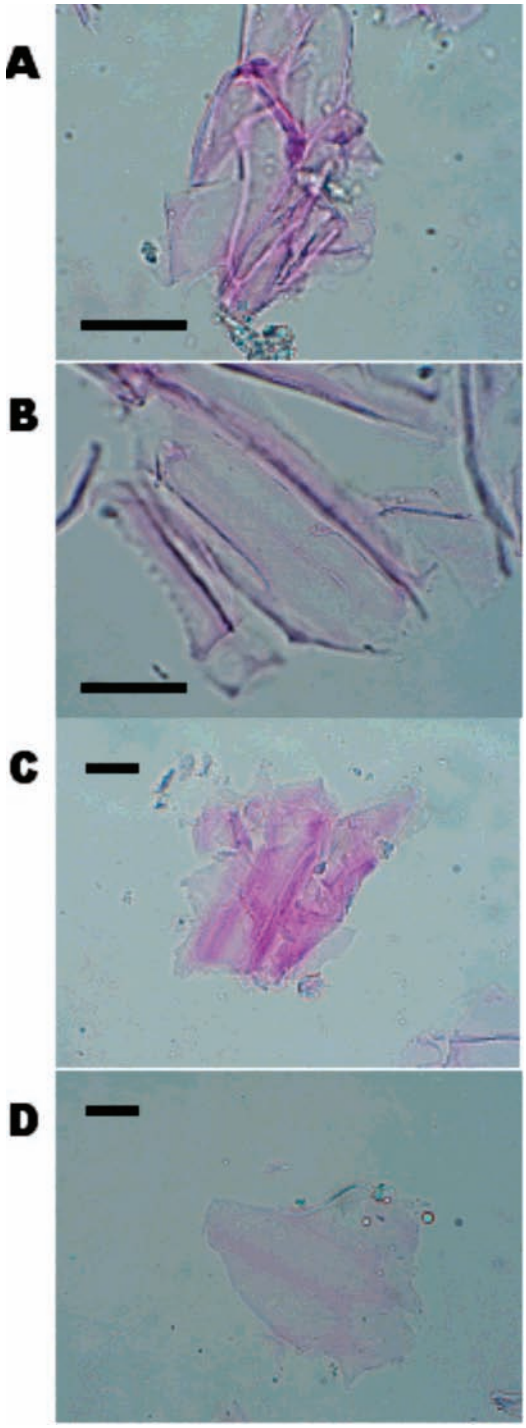

Figure 7. Microscopy of soybean residue after boiling and autoclaving. Microscopy of soybean residue (okara) stained by PAS (**A** and **B**) and acrolein–Schiff reagent (**C** and **D**) after boiling (100 °C, 10 min) or autoclaving (121 °C, 10 min), respectively. The autoclaved soybean residues were stained paler than that of the boiled one. The black bar represents 10 μm.

extraction from the autoclaved okara. The gel was weakened by proteases, and the gel was well-formed in the case of uronic acid, which was also well-extracted (data not shown). As for the food function of each extract, we examined the limited digestive enzymes and ACE. The inhibition activity for ACE would be localized in some proteins. The pepsin and trypsin digestion of extraction C had the strong inhibitory activity for ACE. The activity was strong in the sequence of extraction C > extraction B = extraction D > extraction A. Considering each elution profile of HPLC, the activity was considered to be mainly from digestion of the UV-2 protein of extraction C (**Figure 1**). The high molecular weight protein could be from middle lamella. The proline-rich protein or the arabinogalactan protein was well-known to be in the middle lamella (*14*). Many digested peptides containing proline are often strong ACE inhibitors (*23*, *24*). The protease digestion of soybean meal protein or some peptides of the fermented soybean were also reported (*25*, *26*). Further study on the characteristics of the digestion and the protein of extraction C should be done to clear. The localized protein would have a unique food function. A detailed study will be done in the future.

Many kinds of extracts and components of the soybean are well-studied and used for food materials. Some trials of further use and exploring new food functions from soybean were carried out (*27*−*33*). The stepwise extractions of soybean seed gave a further possibility as food material such as gelation of extraction A and also gave the information of localization or the characteristics of the unique proteins such as extraction C or D. These results would be useful for understanding, improvement, or future application of food processing in using soybean (*27*−*33*). We are continuing to find useful results for new food function for soybeans.

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